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Effect of Wound Lavage on the Intraoperative Contamination Rates during Small Animal Orthopedic Surgery

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Summary

The effect of wound lavage on the intraoperative contamination of the surgical wound during small animal orthopedic surgery was evaluated using a Wilcoxon rank test. Samples were taken during 46 clean and contaminated orthopedic surgeries from the suction tip before the last lavage (n=40) and the surgical wound before (n=46) and after (n=46) the last lavage. For the comparison of suction tip and surgical wound contamination and the estimation of the effect of surgical time on the contamination rates a non-parametric Spearman rank correlation test was used; $p \leq 0.05$ was considered for both tests significant. The surgical wound contamination after the last lavage was not time-dependent (p=0.468 to 0.484) and in clean fracture repair and joint surgeries not associated with suction tip contamination (p=0.277). Coagulase-negative staphylococci were isolated in clean surgeries and coagulase-positive staphylococci in contaminated surgeries. Intraoperative bulb syringe wound lavage did not reduce the contamination rate of the surgical wound in none of the surgical groups (p=0.158 to 0.500).

Keywords: Small animal orthopedic surgery, intraoperative contamination rates, wound lavage

Zusammenfassung

Mit einem Wilcoxon Rank Test wurde der Effekt der Wundspülung auf die intraoperative Wundkontamination bei orthopädischen chirurgischen Eingriffen am Kleintier evaluiert. Es wurden Proben während 46 sauberer und kontaminierter orthopädischer Eingriffe von der Saugspitze vor der letzten Spülung (n=40) und den Operationswunden vor (n=46) und nach (n=46) der letzten Spülung entnommen. Saugspitzen-, Wundkontamination und der Einfluss der Operationszeit auf die Kontaminationsraten wurden mit einem Spearman-Rang-Korrelationstest verglichen; $p \leq 0.05$ galt für beide Tests als statistisch signifikant. Die Wundkontamination nach der letzten Spülung war nicht abhängig von der Operationsdauer (p=0.468-0.484) und bei sauberen Fraktur- und Gelenksoperationen nicht von der Saugspitzenkontamination abhängig (p=0.277). Bei sauberen Eingriffen wurden koagulasenegative Staphylokokken und bei kontaminierten Eingriffen wurden koagulasepositive Staphylokokken isoliert. Die intraoperative Wundspülung mittels einer Ballonspritze konnte die Wundkontamination in keiner der Operationsgruppen reduzieren (p=0.158-0.500).

Schlüsselwörter: Orthopädische Eingriffe beim Kleintier, intraoperative Kontaminationsraten, Wundspülung

1 Introduction

Advanced orthopedic surgical procedures such as complicated fracture repair surgery, tibial tuberosity advancement (TTA) or tibial plateau leveling osteotomy (TPLO) for stabilization of cruciate ligament deficient stifle joints and prosthetic joint implantation (THR) for hip joint dysplasia gain more and more importance in veterinary medicine, improving the quality of life for many patients. Owners spent over 1.3 billion dollars for the treatment of the cranial cruciate ligament rupture in the United States in 2003 (Wilke V.L. et al. 2005).

The importance of advanced orthopedic surgical procedures in veterinary medicine led to the improvement of the measures taken for prevention of surgical site infections (SSI). Despite improvement of prevention, SSI still represents a serious problem in human and veterinary medicine (Weese J.S. 2008, Klouche S. et al. 2010, Nicoll C. et al. 2014). Intraoperative contamination of the surgical wound during human and veterinary orthopedic procedures is estimated to be approximately 0.7-4.0% in human medicine and 2.5-8.1% in veterinary medicine (Weese J.S. 2008, European Centre for Disease Prevention and Control 2012, Lamagni T. 2015, Turk R. et al. 2015).

The financial impact of SSI in human orthopedic surgeries is obvious considering that over 55,000 revisions of total knee osteoarthritis surgeries were performed in 2010 (Bhandari M. et al. 2012). It was shown that the cost of revision surgeries in proximal femoral fractures were twice as high as the cost of the first operation (Thakar C. et al. 2010). The revision cost of a non-infected total hip implant is 1.4 times higher than the primary total hip prosthesis surgery and even 3 to 4 times higher if the implants are infected (Sulco T.P. et al. 1995, Klouche S. et al.1996). In a recent study in veterinary surgery, although the isolation of bacteria had no impact on the likelihood of implant removal after tibial plateau leveling osteotomy (TPLO), the cost for the treatment of surgical site infections in cases where bacteria were isolated was double compared to the treatment cost with a negative culture. Postoperatively, the costs in case of surgical site infection were 7 times higher compared to the cost when surgical site infection did not occur (Nicoll C. et al. 2014).

SSI affects either the superficial incision site or the deep tissue at the operation site and can occur up to 30 days or even up to one year after surgery in the case of implant surgery, and is classified according to superficial, deep, or organ/space infections (Verwilghen D. et al. 2015). The combined interpretation of clinical and laboratory data allows identification of an SSI in one of these categories (Verwilghen D. et al. 2015).

Not all patients with positive intraoperative cultures develop surgical site infection. The virulence of the bacterium and the size of the inoculum are important factors (Bergstrom N. 1994, Fabiano G. 2004).

Staphylococci are most commonly cultured from canine SSI, because of their commensal and opportunistic nature. Based on their coagulase-reaction, staphylococci are divided in two main groups, coagulase-positive and coagulase-negative staphylococci. The coagulase-positive staphylococci are associated with a higher pathogenic potential. They comprise several species, including Staphylococcus aureus, Staphylococcus intermedius and Staphylococcus pseudintermedius (Meakin L.B. et al. 2013, Verwilghen D. et al. 2015). Before 2007, Staphylococcus intermedius was believed to be the major canine staphylococcal pathogen, but molecular reclassification studies have shown that this bacterium is Staphylococcus pseudintermedius. In an investigation into bacterial load and type on veterinary surgeons hands 5.0% of small animal veterinarians were determined to be carriers of Staphylococcus pseudintermedius, whereas no other group of veterinary or human healthcare workers carried this bacterium, which underlines the dissemination of this bacterium in small animal practice (Verwilghen D. et al. 2015). This species has rapidly emerged as the most common cause of canine SSI worldwide, with a recent concerning trend being an apparent marked increase in Methicillin and multidrug resistance. Additionally, Staphylococcus pseudintermedius has biofilm formation ability, leading to an additional resistance in the treatment of implant-associated SSI. Whereas another coagulase-positive staphylococcus, Staphylococcus aureus, having an even greater zoonotic potential and being the leading cause of human SSI, is only occasionally involved in SSI in small animals (Meakin L.B. et. al. 2013, Verwilghen D. et al. 2015). On the other hand, coagulase-negative staphylococci are commensal in a variety of animals, are found commonly in immunocompromised persons, have variable Methicillin und multidrug resistance, but are uncommon causes of SSI. They comprise several species, including Staphylococcus epidermidis, Staphylococcus haemolyticus and Staphylococcus lentus (Weese J.S. 2008; Verwilghen D. et al. 2015).

Owens B.D. et al. in 2009 proposed that eliminating the amount of bacteria in the surgical wound decreases the postoperative infection rate (Owens B.D. et al. 2009). Surgical irrigation is a critical part of the intraoperative process aimed at reducing the risk of SSI by reducing the number of contaminating bacteria (Barnes S. et al. 2014; Ahn D.K. et al. 2015). Watanabe M. and co-workers have shown in 2010 that the risk for postoperative SSI in people undergoing spinal surgery for various reasons could be statistically significantly reduced by intraoperative irrigation of the surgical field with > 2000 milliliter (ml) saline per hour (Watanabe M. et al. 2010). He demonstrated a statistically significant reduction in SSI that was associated with the amount of saline used during irrigation. Watanabe M. and co-workers based their study on a study from Savitz S.I. and co-workers in 1998 (Savitz S.I. et al. 1998) who reported that constant irrigation kept 38.0% of the wounds free of bacteria and prevented identified potential pathogens from propagating on sequential culture in 95.0% of operations. The effect of copious wound lavage in reducing SSI could also be demonstrated in the case of shunt surgery and major hepatobiliary and pancreatic surgery (Hayashi T. et al. 2010, Nikfarjam M. et al. 2014). Nikfarjam M. and co-workers suggested in 2014 that copious intraoperative irrigation reduces the intraoperative wound contamination by reducing the number of bacteria present in the surgical wound.

Trying to define the optimal irrigation volumes which are necessary to prevent surgical site infection, early studies performed on animal models could demonstrate that increasing the saline irrigation volume from 0 to 1,000 ml in 250 ml increment resulted in a steady decrease in the clinical wound infection score (Peterson L.W. et al. 1945). Gainor B.J. and co-workers demonstrated on a bovine muscle model in 1997 that with the increase of the saline irrigation volume from 0.1 liter to 1.0 liter an increased bacterial removal was observed but not after 10 liters (Gainor B.J. et al. 1997, Anglen J.O. 2001). Svoboda S.J. and co-workers in 2006 demonstrated in a study on goats that bulb syringe reduces bacteria equally to pulse lavage when a 3-fold amount of saline was used for irrigation (Svoboda S.J. et al. 2006). Watanabe M.

and co-workers in 2010 suggested intraoperatively wound irrigation every 15 minutes (min) with at least 500 ml saline (Watanabe M. et al. 2010). Epstein N.E. in 2011 suggested an intraoperative irrigation every 15 min with three full bulb syringes (Epstein N.E. 2011).

On the other hand, different studies suggested that the amount of pressure used in irrigation is the key variable to achieve effective wound cleansing (Luedtke-Hoffmann K.A. et al. 2000). In a study performed on an animal model, low pressure irrigation produced by a bulb syringe with approximately 0.5 pound-force per square inch (psi) and high-pressure irrigation using 12- and 35-ml piston syringes through 19-gauge needles (20 and 7 psi, respectively) were compared. It was reported that piston syringe irrigation, at both pressure levels, removed greater amounts of bacteria than the bulb syringe method did (Stevenson T.R. et al. 1976, Luedtke-Hoffmann K.A. et al. 2000) and that higher pressures between 5 and 10 psi were more effective in the reduction of loose necrotic tissue and wound exudate. It was suggested that irrigation pressures above 10 psi protect the wound from gross infections (Luedtke-Hoffmann K.A. et al. 2000). A recent veterinary study suggested an irrigation pressure of 7-8 psi for initial wound irrigation. Irrigation pressures between 25 and 40 psi can cause barotrauma to the wound and are detrimental for the surrounding tissue (Gall T.T. et al. 2010). Owens B.D. and co-workers in 2009 comparing the different irrigation devices, demonstrated that bulb syringe lavage with sterile saline was equal to pulse lavage and superior compared to high pressure lavage in the ability to remove bacteria from an artificially contaminated wound and superior in the rebounding rate of bacterial contamination compared to high but also low pressure lavage (Owens B.D. et al. 2009). Similar, different other animal studies performed in the past showed that wound lavage with a bulb syringe was associated with a lesser tissue and bone damage compared to pulse lavage and a lesser infection rate as it did not cause the spread of the bacteria into deeper tissue (Dirschl D.R. et al. 1998, Crowley D.J. et al. 2007).

Intraoperative contamination of the surgical field can be endogenous or exogenous. In the endogenous contamination of the surgical field, the source of bacterial contamination of the surgical wound is the patient’s commensal microbiota at the surgical site or distant body sites (skin, oropharynx, gastrointestinal tract). In the exogenous contamination of the surgical field, sources of contamination are those originating from the surgical team, the surgical instrumentation and materials used as well as the environment, leading to intraoperative contamination of the surgical field (Haridas M. et al. 2008, Mackain-Bremner A.A. et al. 2008, Verwilghen D. et al. 2015).

A recent veterinary study performed in the Clinic of Small Animal Surgery of the Vetsuisse Faculty of the University of Zurich stated that the suction tip acts as reservoir of bacteria concentrating airborne bacteria. Suction tip cultures revealed bacteria that were associated with normal air and skin flora (Medl N. et al. 2012). In former studies, suction tip bacteria were implicated in deep infections in total hip prosthesis surgeries. The bacteria cultured on revision surgeries of hip prosthesis surgeries were the same as the bacteria cultured during the first surgery from the suction tip and were mainly coagulase-positive staphylococci (Robinson A.H. et al. 1993). Givissis P. and co-workers in 2008 related SSI to suction tip contamination in orthopedic surgical procedures in human medicine, comparing, as Robinson A.H. et al. suggested in 1993, the bacteria isolated from the suction tip with the bacteria isolated from the postoperatively infected surgical wound.

There is no veterinary study, to our knowledge, that investigates the effect of copious bulb syringe wound lavage on the intraoperative contamination rate of the surgical wound. Similar, there is no study that compared intraoperatively suction tip contamination with surgical wound contamination.

The objectives of this study were to determine the contamination rates of the suction tip and the surgical wound at the end of surgery, the association between suction tip and surgical wound contamination, the effect of surgical time on the contamination rates, to classify the bacteria isolated during surgery and, finally, to evaluate the effect of copious intraoperative bulb syringe wound lavage on the amount of bacteria present in the surgical wound at the end of surgery.

Our hypothesis was that the suction tip contaminates the surgical wound (Robinson A.H. et al. 1993, Bernard L. et al. 2004, Givissis P. et al. 2008, Medl N. et al. 2012), higher contamination of the surgical wound occurs at the end of surgery (Greenough C.G. et al. 1986, Berbari E.F. et al. 1998, Clarke M. et al. 2004, Ahn D.K. et al. 2015). Further, we assumed middle grade contaminations and contaminations with bacteria other than coagulase-negative staphylococci are associated with increased risk of clinical SSI (Turk R. et al. 2015, Ahn D.K. et al. 2015, Verwilghen D. et al. 2015). Combined bulb syringe irrigation with copious wound lavage at the end of surgery can significantly reduce the number of bacteria left in the surgical wound before closure (Svoboda S.J. et al. 2006).

2 Material and methods

2.1 Inclusion criteria and group assignment

Signalment (species, age, breed, gender and castration status), type of surgery (fracture repair, joint surgery, THR surgery), duration of each surgery in min and number of surgery (first or revision surgery) were noted. The duration of each surgery was counted from the time the surgical incision was performed till the time after the last surgical wound sample was taken from the surgical wound. The duration of each surgery was round up to 5 min intervals.

All surgical procedures included in this study (n=46, table 1) were performed in the Clinic of Small Animal Surgery of the Vetsuisse Faculty of the University of Zurich, on client-owned dogs and cats and were divided into two main groups: (1) fracture repair and joint surgeries (n=37) and (2) THR (n=9) surgeries. Based on the risk index for SSI (Weese J.S. 2008), the surgeries were divided further into clean and contaminated surgeries as follow: (1) clean fracture repair and joint surgeries (n=30; 65.2%), (2) clean THR surgeries (n=6; 13.0%), (3) contaminated fracture repair and joint surgeries (n=7; 15.2%), and (4) contaminated THR surgeries (n=3; 6.5%). Endoscopic procedures, open fractures and procedures where an obvious break in aseptic technique occurred during surgery were excluded from this assignment.

Surgical groups	Frequency n	Percent
Clean fracture repair and joint surgeries	30	65.2%
Clean THR surgeries	6	13.0%
Contaminated fracture repair and joint surgeries	7	15.2%
Contaminated THR surgeries	3	6.5%
Total cases	46	100.0%

Table 1. Surgical groups

2.2 Preoperative Preparation

The anesthetic and preparation protocol was standardized and was the same for all animals. General anesthesia was induced with individual intravenous premedication. All animals were intubated and anesthesia was maintained in all cases with Isoflurane in oxygen. At induction, cefazolin (Fezol, Teva Pharma AG, Aesch, Switzerland) 22 milligrams per each kilogram body weight were administrated intravenously as a prophylactic preoperative antibiotic and repeated every 90 min during anesthesia. The animals were clipped and aseptically prepared for surgery with a povidone-iodine disinfectant (Betadine, Mundipharma medical company, Basel, Switzerland). All animals were draped using impervious drapes (Custom sterile, care fusion, chateaubriand, France). The first layer was applied in a four drape mode, followed by a second layer covering the whole animal. In THR surgeries the skin in the area of the surgical incision was covered with a special adhesive iodophore impregnated drape (Ioban EZ antimicrobial incise drapes, 3M Healthcare AG, Switzerland) additional to the standard surgical draping. No ventilation system was used. The surgical team was prepared and dressed in a standard manner wearing surgical scrubs, impervious disposable gowns, caps, masks, latex surgical gloves and booties. European College of Veterinary Surgeons (ECVS) Diplomates and/or ECVS Residents, performed the surgeries under direct supervision. Signalment, type and duration of each surgery were recorded.

2.3 Set of the surgical suction

Commercially available disposable plastic suction tips (Kendall Argyle Yankauer, Tyco Health Care, Gosport, UK) connected to latex tubing were used throughout each surgery. The suction tip had the same diameter over the last 3 centimeter (cm) and two marker holes at the side. The tip of the suction was cut before surgery to ensure that the two marker holes were removed. The diameter of the suction tip, after the two marker holes were removed, and the suction tip sample taken remained the same. The surgical suction was used in an intermittent manner- the suction was turned off using a plastic clamp to stop the airflow when not in use, although the former study in our clinic (Medl N. et al. 2012) could not prove a significant difference in the numbers of bacteria isolated from the suction tip during continuous or intermittent suction. The surgical suction apparatus was installed as close as possible to the surgical field during each surgery. The suction was set at a negative pressure of 200 cm of water.

2.4 Intraoperative wound lavage

Sterile Ringer’s lactate was used as irrigation fluid, proven to be less traumatic than normal saline on connective tissue, preserving tissue healing (Greene C.E. 2006). Wound lavage was performed throughout each surgery with a sterile catheter tip bulb type irrigation syringe of 60 ml (Kendall Covidien, Wollerau, Schweiz) with 500 ml of sterile irrigation fluid every 15 min ensuring continuous, low-pressure wound lavage of approximately 0.5 psi (Stevenson T.R. et al. 1976, Luedtke-Hoffmann K.A. et al. 2000). At the end of each surgery a last lavage with 500 ml sterile Ringer’s lactate was performed.

2.5 Specimen collection

Surgical samples were taken from December 2010 to April 2012, always by the same investigators (KB, PMM) during 46 orthopedic surgical procedures in the following manner:

The swab from the surgical wound at the end of the surgery just before the last lavage (n= 46 surgeries) was taken in a circular manner from the deepest point of the surgical wound: around the implants, the bone and joint surface and posterior muscle layer to the subcutis. At the same time, a sample of the plastic, disposable suction tip (n=40 surgeries) was taken in the following manner: the plastic suction tip was disconnected from the latex tubing, and approximately 5 millimeter of the suction tip was cut off using a new sterile set of scissors and forceps and new sterile gloves for the sampling. After the sampling the suction tip was reconnected to the latex tubing. Finally, the last swab from the surgical wound was taken after the last lavage (n=46) from the deepest point of the surgical wound to the subcutis directly after the aspiration of all the fluids present in the surgical wound and after the suction tip was completely removed just before closure.

2.6 Bacteriological analysis

The suction tip and surgical wound samples collected during the surgeries were immediately transferred into tryptic soy broth (TSB, Oxoid, Pratteln, Switzerland). After incubation for 24 hours at 37 degree Celcius (°C) after atmospheric conditions, 100 microliter aliquots were plated onto Columbia blood agar, containing 5.0% defibrinated sheep blood and Gassner agar (Oxoid). Plates were incubated aerobically at 37°C for 24-48 hours and were examined for bacterial growth after 24 and 48 hours.

Differentiation of culture isolates was performed by standard bacteriological procedures using gram staining, catalase test, oxidase test, oxidation-fermentation test, coagulase activity and growth performance on different agar media (Markey B. et al. 2013). Identification was completed by using the API 20E systems according to the manufacturer's instructions (bioMérieux, Geneva, Switzerland).

2.7 Statistical analysis

Data analysis was performed using statistical software (Microsoft Excel und IBM SPSS Statistics Version 22). Factors analyses included patient factors and surgical factors.

The non-parametric Spearman rank correlation was used to detect the association between suction tip and surgical wound contamination and for the detection of the association between the duration of surgery and the maximal contamination of the surgical wound and the suction tip. A non-parametric Mann Whitney U test was used for comparison of the maximal contamination of the wound and the suction tip between the different patient groups. The Wilcoxon rank test was used for comparison of the maximal contamination of the surgical wound before and after the last lavage. Statistical significance was set at a statistical probability (p) value of equal or less to 0.05. Positive association was set at a correlation coefficient value (rs) equal or greater than 0.30 ($rs \geq 0.30$), with values equal or greater than 0.50 ($rs \geq 0.50$) considered as strong positive association.

A non-parametric Kruskal Wallis test was used for comparison of the maximal contamination of the suction tip and the surgical wound for all surgical groups. Due to a significant Kruskal Wallis test, the overall contamination of the surgical wound and the suction tip was further compared separately for each surgical group, in total 6 times, performing a Mann Whitney U test with a p-value set at ≤ 0.0083 (0.05/6) according to the Bonferroni corrected alpha error niveau.

3 Results

3.1 Procedures and animal population

Procedures included in our study (table 2) were clean fracture repair (n=14; 30.4%) and clean joint surgeries (n=16; 34.8%), contaminated fracture repair and contaminated joint surgeries (n=7; 15.2%), clean THR surgeries (n=6; 13.0%) and contaminated THR surgeries (n=3; 6.5%).

Kind of surgery		**Frequency n**	**Percent**
Clean fracture repair		14	30.4%
Clean joint surgeries		16	34.8%
TTA	8		
FCP	2		
Medial epicondylitis	2		
TPO	1		
FHO	1		
ISJL	1		
PIN	1		
Clean THR		6	13.0%
Contaminated fracture repair		3	6.5%
Contaminated joint surgeries		4	8.7%
TTA	2		
TTT-sulcoplasty	1		
Patella tendon repair	1		
Contaminated THR		3	6.5%
Total cases		46	100.0%

Table 2. Kind of surgery

Surgeries were performed on 32 dogs and 14 domestic short-haired cats. The dogs had a mean age of 4.56 +/- 3.29 years; 17 of the dogs were female (10 castrated) and 15 were male (5 castrated). Pure breed dogs (n=22), mainly Labrador Retrievers (n=5) were overrepresented. Another 10 breeds of dog were presented.

The cats had a mean age of 4.42 +/- 4.31 years; the number of female cats (n=8) was higher than the number of male cats (n=6) with most of the female cats (n=6) and all of the male cats (n=6) being neutered.

3.2 Surgical wound and suction tip contamination

Suction tip contamination before the last lavage occurred in 7 of 26 clean fracture repair and joint surgeries (26.9%) and in 5 of 7 (71.4%) contaminated fracture repair and joint surgeries. In the group of clean THR surgeries no suction tip contamination occurred; in the group of contaminated THR surgeries contamination of the suction tip before the last lavage occurred in 2 of 3 (66.7%) surgeries. Surgical wound contamination before the last lavage occurred in 1 of 30 clean fracture repair and joint surgeries (3.3%) and in 5 of 7 contaminated fracture repair and joint surgeries. The clean THR surgeries had surgical wound contamination before the last lavage in 1 of 6 (16.7%) surgeries, for the contaminated surgeries the contamination rate of the surgical wound before the last lavage was 100.0% (n=3/3).

Surgical wound contamination after the last lavage occurred in 2 of 30 (6.7%) clean fracture repair and joint surgeries and in 4 of 7 (57.2%) contaminated fracture repair and joint surgeries. For the clean THR surgeries the surgical wound contamination after the last lavage occurred in 1 of 6 (16.7%) surgeries. For the contaminated THR surgeries surgical wound contamination occurred in all surgeries (n=3/3; 100.0%).

3.3 Bacteriological findings

Bacteria were isolated from 10 of 30 clean surgeries (33.3%), 1 of 6 clean THR surgeries (16.7%), 5 of 7 contaminated surgeries (71.4%) and in 3 of 3 contaminated THR surgeries (100.0%). In 2 contaminated surgeries 2 different bacterial species were isolated at the same time.

In the surgical wound of clean surgeries only coagulase-negative staphylococci occurred. Similarly, the main bacteria isolated from the suction tip of the clean surgeries were coagulase-negative staphylococci (table 3). All the contaminations were low-grade (table 4). In the contaminated surgeries we isolated mainly coagulase-positive staphylococci from the surgical wound and the suction tip (table 3). Except for three middle-grade contaminations with coagulase-positive staphylococci, all the others were low-grade (table 4).

In clean THR surgeries the surgical wounds were contaminated with Ralstonia sp. only in one primary surgery. In contaminated THR surgeries surgical wound and suction tip were mainly contaminated with coagulase-positive staphylococci (table 3). In both, clean and contaminated THR surgeries, all contaminations were middle-grade (table 4).

Isolated bacteria	SURGICAL GROUPS									
	clean surgeries		clean THR surgeries		contaminated surgeries		contaminated THR surgeries		Total	
	St	Sw b/a	St	Sw b/a	St	Sw b/a	St	Sw b/a	St	Sw b/a
Staphylococci coagulase-negativ	5	1 / 2	0	0 / 0	0	0 / 0	0	0 / 0	5	1 / 2
Staphylococci coagulase-positiv	0	0 / 0	0	0 / 0	4	3 / 3	2	2 / 2	6	5 / 5
Micrococci	1	0 / 0	0	0 / 0	0	0 / 0	0	0 / 0	1	0 / 0
Bacillus	1	0 / 0	0	0 / 0	1	1 / 1	0	0 / 0	2	1 / 1
Escherichia coli	0	0 / 0	0	0 / 0	1	1 / 0	0	0 / 0	1	1 / 0
Enterococcus	0	0 / 0	0	0 / 0	1	1 / 0	0	0 / 0	1	1 / 0
Klebsiella	0	0 / 0	0	0 / 0	0	0 / 1	0	0 / 0	0	0 / 1
Ralstonia	0	0 / 0	0	1 / 1	0	0 / 0	0	0 / 0	0	1 / 1
Enterobacter	0	0 / 0	0	0 / 0	0	0 / 0	0	1 / 1	0	1 / 1

St= suction tip
Sw b/a= surgical wound before last lavage / after last lavage

Table 3. Isolated bacteria from the suction tip and the surgical wounds within the surgical groups

3.4 Statistical analysis

Performing a Mann Whitney U test with consideration of the Bonferroni corrected alpha error niveau ($p<0.0083$ statistically significant) we could prove that the contamination of the surgical wound in the group of clean fracture repair and joint surgeries was statistically significantly different compared to the contaminated surgeries ($p=0.001$). There was no significant difference in the suction tip contamination between the different surgical groups ($p=0.021$ to $p=0.398$). In elective THR surgeries, no suction tip contamination occurred nor a difference in surgical wound contamination compared to the contaminated surgeries occurred ($p=0.020$ to $p=0.060$).

For clean fracture repair and joint surgeries a Spearman rank correlation test with $p\leq0.050$ considered statistically significant, confirmed that there was no statistically significant association between the contamination of the suction tip before and the surgical wound after the last lavage (p=0.277; table 4). In the contaminated surgeries an overall statistically strong association between suction tip contamination before and surgical wound contamination after the last lavage was demonstrated (p=0.009; table 4). Testing this significance separately for coagulase-negative staphylococci, there was no association between the contamination of the suction tip before and the surgical wound after the last lavage with coagulase-negative staphylococci for the clean fracture repair and joint surgeries (p=0.318), although for the contaminated surgeries the association between suction tip contamination before and surgical wound contamination before and after the last lavage with coagulase-positive staphylococci was strong (rs=0.839) and statistically significant (p=0.009).

Contamination rates		KIND OF SURGERY							
		clean surgeries		clean THR surgeries		contaminated surgeries		contaminated THR surgeries	
			%		%		%		%
Contamination of surgical wound **before** last lavage (cSwb)	below limit of detection	29	96.7%	5	83.3%	2	28.6%	0	0.0%
	low-grade	1	3.3%	0	0.0%	4	57.1%	0	0.0%
	middle-grade	0	0.0%	1	16.7%	1	14.3%	3	100.0%
	high-grade	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	missing data	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	TOTAL	30	100.0%	6	100.0%	7	100.0%	3	100.0%
		p=.282 (cSwa/b)		**p=.5 (cSwa/b)**		**p=.158 (cSwa/b)**		**p=.5 (cSwa/b)**	
Contamination of surgical wound **after** last lavage (cSwa)	below limit of detection	28	93.3%	5	83.3%	3	42.9%	0	0.0%
	low-grade	2	6.7%	0	0.0%	3	42.9%	0	0.0%
	middle-grade	0	0.0%	1	16.7%	1	14.3%	3	100.0%
	high-grade	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	missing data	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	TOTAL	30	100.0%	6	100.0%	7	100.0%	3	100.0%
		p=.277 (cSwa/st)				**p=.009 (cSwa/st)**			
Contamination of **suction tip wound before** last lavage (cSt)	below limit of detection	19	73.1%	4	100.0%	2	28.6%	1	33.3%
	low-grade	7	26.9%	0	0.0%	4	57.1%	0	0.0%
	middle-grade	0	0.0%	0	0.0%	1	14.3%	2	66.7%
	high-grade	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	missing data	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	TOTAL	26	100.0%	4	100.0%	7	100.0%	3	100.0%

Table 4. Summary of the contamination rates and the statistical associations between suction tip and surgical wound contaminations in the different surgical groups

Finally, performing a Wilcoxon rank test with $p \leq 0.050$ statistically significant, we could prove that there was no statistically significant decline in the overall contamination rate of the surgical wound after the last lavage (p=0.500). The contamination of the surgical wound in the group of clean and contaminated fracture repair and joint surgeries did not significantly change after the last lavage (p=0.282 and p=0.158 respectively; table 4 and figures 1.1 and 1.2), as well as in the groups of clean and contaminated THR surgeries (p=0.500 respectively for both surgical groups; table 4 and figures 2.1 and 2.2).

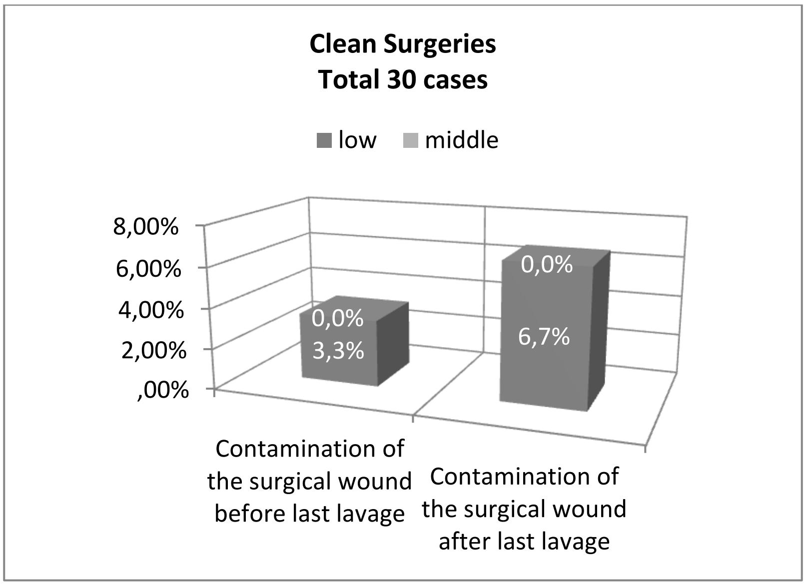

Figure 1.1. Comparison of surgical wound contamination before and after the last lavage for evaluation of the effect of wound lavage in clean fracture repair and joint surgeries

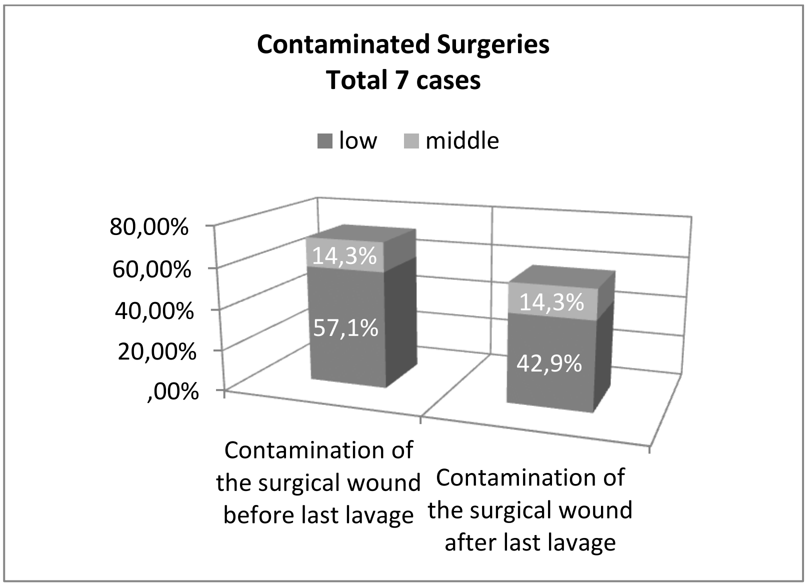

Figure 1.2. Comparison of surgical wound contamination before and after the last lavage for evaluation of the effect of wound lavage in contaminated fracture repair and joint surgeries

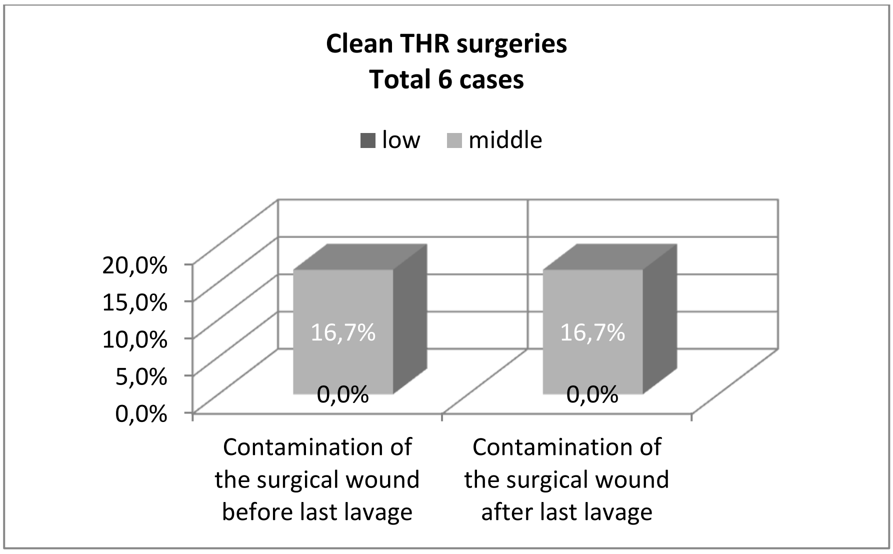

Figure 2.1. Comparison of surgical wound contamination before and after the last lavage for evaluation of the effect of wound lavage in the group of clean THR surgeries

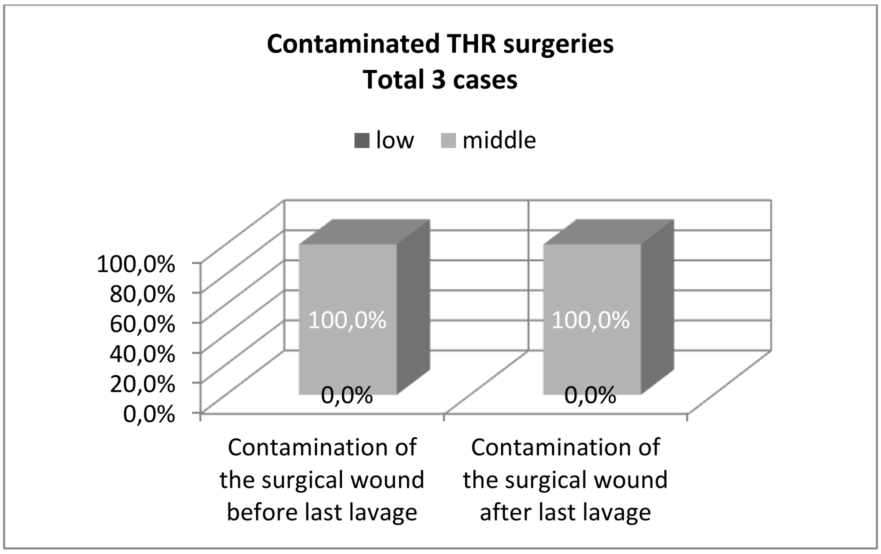

Figure 2.2. Comparison of surgical wound contamination before and after the last lavage for evaluation of the effect of wound lavage in the group of contaminated THR surgeries

Finally, no significant association between species and contamination rates of the suction tip (dog and cats p=0.132 to 0.163), surgical wound before (dogs and cats p=0.163 to 0.253) and surgical wound after the last lavage (dogs and cats p=0.280 to 0.846) were found. Similarly, no difference was found between castrated and not castrated animals (p=0.480 to 1.000) and between male and female (p=0.208 to 1.000); $p \leq 0.050$ was considered statistically significant.

3.5 Time dependence

The mean duration of all procedures was 123.48 min (median 95.00 min, range from 25.00 to 375.00 min +/- standard deviation (SD) 62.46).

Performing a Spearman rank correlation test we could demonstrate the absence of any statistically significant association between surgical time and maximal contamination of the suction tip in clean ($p=0.305$) and contaminated fracture repair and joint surgeries ($p=0.458$). No suction tip contamination occurred in elective THR surgeries. In contaminated THR surgeries, there was no association between suction tip contamination and surgical time ($p=0.334$); $p \leq 0.050$ was considered statistically significant.

Similarly, there was no statistically significant association between surgical time and surgical wound contamination after the last lavage for clean ($p=0.468$) and contaminated ($p=0.484$) fracture repair and joint surgeries. Also in THR surgeries, surgical wound contamination was not time-dependent ($p=0.139$).

4 Discussion

4.1 Contamination rates

SSI does not develop in all patients with intraoperative contamination proven as positive intraoperative wound cultures. Most wounds are contaminated but contamination with a low bacterial concentration does not lead to infection (Krizek T.J. et al. 1975, Fabiano G. 2004, Ahn D.K. et al. 2015). In the present study, in clean fracture repair and joint surgeries only low-grade contaminations occurred. Middle-grade contaminations occurred mainly in contaminated surgeries. The contamination rates of the surgical wound at the end of surgery were similar to those reported in human studies where contamination rates between 4.1%-15.2% for THR surgeries (Davis N. et al. 1999, Clarke M. et al. 2004, Jonsson E.Ö. et al. 2014), 17.0% for total knee prosthesis surgeries (Jonsson E.Ö. et al. 2014) and 7.6%-10.1% for neurosurgical procedures (Ahn D.K. et al. 2015) were reported.

Although the air, the persons in the surgical room and the skin of the patients are potential sources of surgical wound contamination many studies have suggested that the suction tip can be an additional source of surgical wound contamination (Robinson A.H. et al. 1993, Bernard L. et al. 2004, Givissis P. et al. 2008, Medl N. et al. 2012). The contamination rate of the suction tip was similar to those reported in other human and veterinary studies, where contamination rates of the suction tip were 54.0% for elective fracture repair and spine surgeries (Givissis P. et al. 2008), 68.0% for clean, clean-contaminated and contaminated surgeries (Sturgeon C. et al. 2000), 11.4%-37.0% for elective THR surgeries (Greenough C.G. 1986, Robinson A.H. et al. 1993, Davis N. et al. 1999) and 0.0%, 20.0% and 44.0% for clean orthopedic surgeries (Medl N. et al. 2012). Suction tip contamination between the surgical groups was not significantly different ($p=0.018$ to 0.398), except of the group of clean THR surgeries, where no suction tip contamination occurred. The contamination of the surgical wound was significantly different between clean and contaminated surgeries ($p=<0.0083$). In clean fracture repair and joint surgeries suction tip contamination occurred mainly in joint surgeries where no surgical wound contamination occurred. This lack of association between suction tip and surgical wound contamination in clean fracture repair and joint surgeries could be demonstrated statistically and was significant in contrast to the suggestions of former studies (Robinson A.H. et al. 1993, Bernard L. et al. 2004, Givissis P. et al. 2008, Medl N. et al. 2012).

Finally, similar to another study with similar patient size (Medl N. et al. 2012, Tobias K.M. et al. 2012) the examined patient factors (species, gender, castration) did not influence suction tip and surgical wound contamination.

4.2 Bacterial populations

Based on the former study performed in our clinic, where no aerobic bacteria were found, even using specialized methods for anaerobic cultivation (Medl N. et at. 2012), we exclusively cultured aerobic organisms. The organisms we cultured were associated with normal air and skin flora and were similar to those previously described in literature (Weese J.S. 2008, Lamagni T. et al. 2015, Turk R. et al. 2015, Norton T.D. et al. 2014 and Jonsson E.Ö. et al. 2014).

In contrast to former studies (Sturgeon C. et al. 2000, Givissis P. et al. 2008, Medl N. et al. 2012), in the present study coagulase-positive staphylococci were cultivated exclusively from contaminated surgeries.

The main bacteria which contaminated the suction tip in clean fracture repair and joint surgeries were coagulase-negative staphylococci. The main bacteria which contaminated the surgical wound in clean fracture repair and joint surgeries were also coagulase-negative staphylococci but were not statistically associated with the coagulase-negative staphylococci isolated from the suction tip in clean fracture repair and joint surgeries (p=0.318). The main bacteria which contaminated the surgical wound and suction tip in contaminated surgeries were coagulase-positive staphylococci. The coagulase-positive staphylococci isolated from the surgical wound in contaminated surgeries were statistically significant associated with the coagulase-positive staphylococci isolated from the suction tip in contaminated surgeries (p=0.009). We believe that the significant association between suction tip and surgical wound contamination with coagulase-positive staphylococci in contaminated surgeries should be attributed to the contamination of the suction tip from the already contaminated surgical wound with coagulase-positive staphylococci and not the opposite.

4.3 Effect of wound lavage

Wound irrigation is thought to be the principal method to control bacterial contamination during surgery (Ahn D.K. et al. 2015). Based on the statement that the virulence and the size of the inoculum size are important factors in the development of SSI (Fabiano G. 2004, Jonsson E.Ö. et al. 2014), we defined the isolated bacteria and the bacterial load of the surgical wounds to optimize the assessment of the effect of wound lavage in surgical wounds with different risk index for the development of SSI (Weese J.S. 2008). In former studies, middle-grade contaminations and contaminations with coagulase-positive staphylococci were associated with increased rates of SSI (Krizek T.J. et al. 1975, Fabiano G. 2004, Turk R. et al. 2015, Verwilghen D. et al. 2015). Despite copious irrigation with 500 ml Ringer`s lactate (Watanabe M. et al. 2010), surgical wound contamination after the last lavage was statistically not significant different (p>0.050) compared to surgical wound contamination before the last lavage in all surgical groups, independent of the virulence and the concentration of the isolated bacteria. This is in agreement with former studies where bulb syringe lavage had no effect on the incidence of surgical site infection, based on the little effect of bulb syringe lavage on the bacterial count of artificially contaminated animal wounds and elective human neurosurgical procedures (Luedtke-Hoffmann K.A. et al. 2000, Chatterjee J.S. 2005, Crowley D.J. et al. 2007, Ahn D.K. et al. 2015).

4.4 Time dependence

In human studies an increase in the contamination of the suction tip was reported during surgery, with the time cut set after 60 min (Givissis P. et al. 2008). In a veterinary study where the time cut was set at 120 min no influence of time in the contamination of the suction tip could be reported as they failed to demonstrate increased contamination rates of the suction time after that time (Sturgeon C. et al. 2000). Medl N. and co-workers showed in 2012 that there was no influence of time in the suction tip contamination during clean orthopedic surgical procedures performed on dogs and cats. She reported first contaminations of the suction tip as fast as 20 min after the beginning of the surgery, but failed to give a safe time cut after which suction tip contamination occurred (Medl N. et al. 2012). Similarly, in our study we could found no increase of suction tip contamination with time.

Further, in contrast to human studies which set the time cut after which surgical wound contamination increased at 90 min (Byrne A.M. et al. in 2007) and 130 min (Jonsson E.Ö. et al. in 2014), in our study we did not observe an increase in the surgical wound contamination rate with time.

Contamination of the surgical wound before the last lavage in clean fracture repair and joint surgeries occurred only in one surgery. This clean fracture repair surgery performed on a cat was one of the longer lasting surgeries, with an operation time of over 270 min. Surgical wound contamination after last lavage occurred in two surgeries, each lasting 90 min and no surgical wound contamination after the last lavage occurred in the surgery of the same surgical group lasting 270 min with positive surgical wound contamination before the last lavage. This would explain the absence of time-dependence after the last lavage in the group of clean fracture repair and joint surgeries.

In clean THR surgeries, surgical wound contamination did not increase with surgical time, because only in one clean THR surgery, lasting 120 min, surgical wound contamination before and after last lavage occurred and not in the clean THR surgeries lasting 120, 150, 180, 210 and 375 min. Similarly, in contaminated fracture repair and joint surgeries, surgical wound contamination did not increase with prolonged operation time, surgeries lasting 150 min and 210 min had equal or even lower surgical wound contamination than surgeries lasting 90 min. and 120 min.

The absence of time-dependence in surgical wound contamination after the last lavage is in contrast to former studies (Clarke M. et al. 2004, Weese J.S. 2008) although a recent veterinary study demonstrated the absence of any association between duration of surgery and incidence of SSI (Turk R. et al. 2015).

4.5 Limitations of the study

The size of the different surgical groups was small in the present study. Additionally, in the group of clean surgeries the incidence of suction tip and surgical wound contamination was low and only aerobic cultures were performed. Former studies related the low rate of intraoperative contaminations in elective surgeries to the small number of bacteria on the suction tip, the short culturing time, but also to the absence of tissue cultures (Robinson A.H. et al. 1993, Jonsson E.Ö. et al. 2014). Bernard L. and co-workers in 2004 could not always associate positive cultures of the surgical wound obtained during clean orthopedic surgery with post-operative infection. They concluded that cultures performed during clean orthopedic surgery may not be useful in predicting postoperative infection (Bernard L. et al. 2004). Another study detected much higher contamination rates by polymerase chain reaction; 9 versus 2 from the enriched culture (Clarke M. et al. 2004). A recent study was in agreement with those conclusions, reporting that tissue cultures were more sensitive and specific than superficial swabs in the prediction of postsurgical joint infection (Aggarwal V.K. et al. 2013). For this reasons, we failed in elective THR surgeries to define the time-dependence of suction tip contamination and the association between suction tip and surgical wound contamination.

A recent human study demonstrated the superiority of the pulse lavage compared to bulb syringe lavage for similar irrigation volumes in human spine surgery (Ahn D.K. et al. 2015), although other studies have shown that different irrigation devices, other than bulb syringe lavage, were not proved to be superior in the decontamination of the surgical wound and in the prevention of SSI, especially when bulb syringe lavage is combined with higher irrigation volumes (Peterson L.W. et al. 1945, Dirschl D.R. et al. 1998, Svoboda S.J. et al. 2006, Crowley D.J. et al. 2007). The lack of a control group where higher irrigation volumes or different irrigation devices were used is another weak point of this study and precludes objective conclusions about the optimal irrigation volume or device.

Finally, the goal of this study was not the incidence of SSI itself, but the investigation of the effect of last lavage in the bacterial contamination at the end of surgery making suggestions and not drawing definite conclusions about the incidence of SSI.

4.6 Conclusions

Surgical wound contamination after the last lavage did not increase with prolonged surgical time and was significantly higher in the group of contaminated fracture repair and joint surgeries compared to the clean surgeries. In the group of clean fracture repair and joint surgeries only low-grade contaminations occurred with bacteria of low virulence, mainly coagulase-negative staphylococci. In the group of contaminated fracture repair and joint surgeries and in all THR surgeries middle-grade contaminations occurred and the isolated bacteria were of increased virulence, other than coagulase-negative staphylococci. Low-grade contaminations with coagulase-negative staphylococci are associated rather with subclinical than clinical SSI, whereas middle-grade contaminations with bacteria, other than coagulase-negative staphylococci, are associated rather with clinical SSI (Turk R. et al. 2015, Ahn D.K. et al. 2015).

The contamination of the suction tip was similar between the surgeries and did not increase with prolonged surgical time. In the present study we could demonstrate that suction tip contamination was not time-dependent and not associated with surgical wound contamination in clean fracture repair and joint surgeries, in contrast to the suggestion of former human studies (Robinson A.H. et al. 1993, Bernard L. et al. 2004, Givissis P. et al. 2008).

Copious bulb syringe lavage at the end of surgery with 500 ml sterile Ringer's Lactate solution was unable to reduce surgical wound contamination in all surgical groups, independent from the virulence of the isolated bacteria, the contamination rate, and the risk index for the development of SSI. Former studies reported efficient wound lavage after increasing the irrigation volume to 1 liter (Peterson L.W. 1945, Gainor B.J. et al. 1997) in contaminated fractures, even volumes of 6-10 liter were suggested reasonable (Anglen J.O. et al. 2001). A recent study demonstrated that even irrigation volumes over 1 liter performed with a bulb syringe were ineffective, but not when lavage was performed with pulsatile irrigation devices (Ahn D.K. et al. 2015).

It is absolutely necessary to reduce intraoperative surgical wound contamination during primary, elective orthopedic surgery in order to prevent SSI (Ahn D.K. et al. 2015). Transferring the conclusions of a recent review from human surgery to the present study (Barnes S. et al. 2014), we agree that optimal irrigation volumes still remain undefined. Further investigation with larger sample size for each surgical group, different irrigation devices (Ahn D.K. et al. 2015) or higher irrigation volumes (Peterson L.W. et al. 1945, Dirschl D.R. et al. 1998, Svoboda S.J. et al. 2006, Crowley D.J. et al. 2007, Ahn D.K. et al. 2015) and possibly more specific and sensitive culture methods (Clarke M. et al. 2004, Aggarwal V.K. et al. 2013, Jonsson E.Ö. et al. 2014) are recommended.

5 Abbreviations

°C	degree Celcius
cm	centimeter
cSt	suction tip contamination before the last lavage
cSwa/b	Wilcoxon rank test correlation between surgical wound contamination before and surgical wound contamination after the last lavage
cSwa/st	Spearman rank order correlation between the contamination rates of the surgical wound after the last lavage and the suction tip before the last lavage
ECVS	European College of Veterinary Surgeons
FCP	fragmented coronoid process surgery
FHO	femurhead and –neck exsitional arthroplasty
ISJL	ileosacral joint luxation reposition surgery
KB	med.vet. Katerine Besserer
min	minutes
ml	milliliter
n	sample size
PIN	pectineus/ileopsoas myotomy with hip joint capsule neurotomy
PMM	Prof. Dr. med. vet. P.M. Montavon
psi	pound-force per square inch
p	statistical probability
rs	Spearman correlation coefficient
SD	standard deviation
SSI	surgical site infection
sp	species
St	suction tip
Sw b/a	surgical wound before last lavage / after last lavage
THR	total hip replacement surgery
TPLO	tibial plateau leveling osteotomy

TPO	triple pelvic osteotomy
TTA	tibial tuberositas advancement
TTT-sulcoplasty	tuberositas tibiae transposition with sulcoplasty
Z-value	normal distribution value

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